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Cilia in pancreatic development and function: endocrine sensors as well?

Dr Seuss had it right,
as he drew through the night.
A cilia will do,
on Cindy-Lou *Who*—
or better yet, two![1]

He pre-dated, by years
Observation by peers
That cilia exist
In the most unlikely places
Such as the ducts and islets
Of our own pancreases.

In 1962, we saw the first report of cilia in the chick pancreas, at the dawn of the age of electron microscopy. In reports through the 1960s, single cilia were seen on the apical surface of pancreatic duct epithelial cells, on acinar cells, and on the interstitial cells and beta cells of the islets—in gerbils, toads, pigeons, and by 1971 in man. It was difficult to argue that a single cilium per duct epithelial cell was capable of promoting fluid movement through the pancreatic duct system, so their function remained unknown. Ultrastructural differences between pancreatic cilia and classic kinocilia led Bockman *et al*[2] to propose in 1986 that these cilia were sensory in nature. There was virtually no further scientific investigation of pancreatic cilia until 2004, when Cano *et al*[3] reported that embryonal exocrine pancreatic development was dependent upon the normal maturation and expression of primary cilia, as evidence by lack thereof in the orpk mouse. The breakthrough was realization that the molecular defects of the polycystic kidney diseases affected the pancreas as well. Specifically, the cystic kidney disease proteins polycystin-1, polycystin-2, and fibrocystin that cause dominant and recessive forms of polycystic kidney disease, respectively localize to cilia or the basal bodies of cilia. In this instance, localization of polycystin-2 is disrupted in orpk mice.

Attention must then be given to the role of primary cilia as mechanosensors for fluid flow (air or water) in the lumina of kidney collecting ducts, and on the apical surfaces of other lumina such as the nasal passages, lacrimal ducts, bile ducts, and even the orbit. The fact that polycystic kidney disease is commonly accompanied by hepatic and pancreatic cysts implies that cilia are important in the development of these organs as well. Witness then the Bardet–Biedl syndrome (BBS), in which mutations in cilia- or basal body-localized proteins lead not only to nephronophthisis but also obesity and diabetes, among other systemic abnormalities.

The report by **Zhang, Davenport *et al***[4] (p. 45) in the current issue of *Lab Invest* thus assumes critical importance, by demonstrating that loss of polaris (*Tg737*), an intraflagellar transport (IFT) protein required for ciliogenesis, on the orpk strain background, causes abnormalities in both the exocrine and endocrine pancreas. Similar to the orpk mice, these $Tg737^{orpk}$ mice develop cystic lesions in the kidneys, cholangitis with biliary and bile duct hyperplasia, hydrocephalus, and skeletal patterning defects. Dilatations of the pancreatic ducts are evident late in gestation and continue to expand postnatally. Shortly after birth, the acini become disorganized, undergo apoptosis, and disappear. There was normal differentiation and distribution of cell types within the islets. However, in these $Tg737^{orpk}$ mice, postnatal fasting leads to much lower levels of blood glucose than in wild-type mice. Challenge of $Tg737^{orpk}$ mutants with a glucose tolerance test led to marked elevations of circulating glucose, evidence of defects in systemic glucose uptake. One must note that Polaris, the protein encoded by the *Tg737* gene, is not only critical for intraflagellar transport but is also part of the hedgehog signaling pathway for insulin production and glucose homeostasis. Consideration must therefore also be given to the potential role of cilia in the endocrine pancreas as sensing and/or regulating blood glucose levels. Whether ciliary defects lead directly to disrupted insulin production by β-cells or manifest alterations in glucose homeostasis elsewhere is now a highly relevant question.

References

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Hyperinsulinemia, fatty acid synthase, and hepatocarcinogenesis: exploring potential links between obesity and cancer

The International Agency for Research on Cancer has now classified the evidence of a causal link between obesity and cancers of the colon, female breast (postmenopausal), endometrium, kidney (renal cell), and esophagus (adenocarcinoma) as 'sufficient'.[1] Given the rising worldwide trend in obesity, one can posit that overeating may become the largest avoidable cause of cancer in nonsmokers (pun not intended). It is therefore of interest that fatty acid synthase (FAS), the key enzyme of *de novo* fatty acid synthesis, has been implicated in carcinogenesis of numerous human malignancies, including breast, colorectal, and prostate carcinomas. Normally, FAS is mainly expressed in the liver, yet the role of hepatocarcinogenesis has not been investigated. There is an increased risk of primary liver cancer in patients with diabetes mellitus, and FAS expression stimulated by hyperinsulinemia and hyperglycemia, a feature of type II diabetes. Insulin is the primary trigger of hepatocarcinogenesis in an endocrine experimental model, whereby a low number of islets of Langerhans are transplanted into the livers of diabetic rats.

In this issue, **Evert *et al***[2] (p. 99) investigated whether FAS is implicated in hepatocarcinogenesis in the rat hepatic islet-cell transplantation model, in comparison to chemically induced hepatocarcinogenesis after *N*-nitrosomorpholine (NNM) treatment in diabetic and normoglycemic rats. Livers of islet-cell-transplanted diabetic rats exhibited preneoplastic clear-cell foci of altered hepatocytes. These foci are characterized by accumulation of excess glycogen and lipids, and an increased proliferative rate, earning them the moniker of foci of atypical hepatocytes (FAH). Virtually all (96–98%) of the FAH, as well as the glycogenotic hepatocellular adenomas and carcinomas arising in this model, showed strong FAS mRNA and protein overexpression. In the NNM-model as well, FAS protein was also overexpressed in the vast majority (87%) of glycogenotic clear-cell foci and glycogenotic neoplasms, in particular in the diabetic animals. FAS overexpression was thus demonstrated to be an early phenonemon in spontaneous, hormonally and chemically induced rat hepatocarcinogenesis. FAS overexpression can be attributed to the local hyperinsulinemia in the transplantation model, and belongs to cellular and metabolic insulinomimetic alterations in the chemical model. This study does not demonstrate a causal relationship between FAS overexpression and hepatocarcinogenesis. Nevertheless, by demonstrating FAS overexpression in clear-cell preneoplastic foci of spontaneously developing, as well as hormonally and chemically induced rat hepatocarcinogenesis, a possible role of FAS in the development of hepatocellular tumors is now implicated.

References

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An expanded role for small proline-rich proteins in epithelial barrier function: effective use of genomic screening

The ability to screen vast portions of the genome for disease-associated alterations in gene expression has created a critical challenge for biomedical science: what to do with genes so identified. The days of 'identify genes, publish findings' were short-lived, much as the early era of electron microscopy (obtain ultrastructural images, publish findings) was short-lived. This is not to diminish the value of compiling databases on gene alterations, but there is compelling need to determine whether alterations in phenotype—molecular or structural, are causally linked or coincidentally associated. In the current issue, **Nozaki *et al***[1] (p. 109) present an exemplary study of how initial genomic screening can open up a highly opportune new area of investigation, with immediate clinical implications. To wit: common bile duct ligation causes obstruction of the biliary tree, which leads to intrahepatic fibrosis, and eventual cirrhosis and liver failure if unremitted. A key feature of biliary tract obstruction is impaired barrier function of the intrahepatic biliary tree, permitting toxic bile salts to leach into hepatic tissues and initiate pro-fibrotic inflammatory cascades. Homozygous negative IL-6 mice (IL-$6^{-/-}$) subjected to common bile duct ligation exhibit greater impairment of barrier function. Screening microarray analysis identified small proline-rich protein 2 (SPRR2) as a candidate gene that is expressed in biliary epithelial cells, is IL-6 responsive, and is potentially related to biliary barrier function. SPRR2 proteins function as structural cross-linking proteins in squamous epithelia such as the skin, helping to form the environmental barrier. Deficient SPRR2 expression in the biliary epithelia of IL-$6^{-/-}$ mice rendered them more susceptible to biliary injury following common bile duct ligation. IL-6 replacement therapy reversed the biliary barrier defect induced by common bile duct ligation, coincident with restoration of SPRR2 expression. *In vitro* studies demonstrated that IL-6/gp130 signaling, mediated primarily by STAT3, stimulated noncoordinate expression of SPRR2 in the biliary epithelium. These studies thus implicate a broader role for SPRR2 in epithelial barrier function beyond the skin, and point towards strategies by which enhancement of SPRR2 expression might ameliorate the grave consequences of epithelial barrier dysfunction.

Reference

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VHL exon deletion screening: finding the 'forme fruste'

Von-Hippel–Lindau (VHL) disease is an autosomal dominant tumor syndrome in which patients develop capillary hemangioblastomas of the brain and spinal cord, renal cell carcinoma, pancreatic islet cell tumors, pheochromocytoma, and endolymphatic sac tumors along with cysts of the kidney and pancreas. The annual incidence is 1 in 36 000–45 000 live births and the trait has a greater than 90% penetrance. Clinical criteria for the disease require at least two hemangioblastomas or typical visceral lesions in the absence of a family history, or a single hemangioblastoma or typical visceral lesion in a patient with a family history. However, the syndrome in its classic form probably represents only a subset of a more diverse nosologic entity that may encompass a wide variety of incomplete or atypical presentations or *'formes frustes'*.

Mutations in the *VHL* tumor suppressor gene, located on the short arm of chromosome 3p25–26, cause the syndrome and sometimes correlate with clinical phenotype. For example, type I VHL includes patients without pheochromocytoma while type 2 VHL includes patients with pheochromocytoma and renal cell carcinoma (type 2A) or pheochromocytoma without renal cell carcinoma (type 2B). Each of these phenotypes is associated with specific germline mutations of the *VHL* gene. Standard molecular methods to determine gene copy number by Southern blot are labor intensive and require large amounts of high-quality DNA and the use of radioisotopes, while fluorescence *in situ* hybridization lacks the sensitivity needed to detect small exon deletions. Improved molecular methods for screening such individuals as well as family members of VHL patients will help clarify the relationship of 'incomplete' phenotypes to *VHL* gene mutations, thus allowing for appropriate genetic counseling and clinical follow-up.

In this issue **Hoebeeck *et al***[1] (p. 24) present a promising technique for the detection of VHL exon deletions using real-time quantitative PCR (Q-PCR). The method uses a quantification strategy based on SYBR Green I detection and normalization using 2 reference genes with a normal copy number (*ZNF80* (3q13.31) and *GPR15* (3q12.1). The choice of primers was also important for accurate discrimination between 1 and 2 exon copies. The rapid Q-PCR method (3.5 h) provided identical results to those obtained by Southern blot. Hence, Q-PCR can provide accurate and sensitive exon deletion screening in routine DNA diagnosis of VHL and in those individuals with incomplete disease manifestations. A similar approach may facilitate screening for other genetic disorders and reveal a wider spectrum of genetic mutations in familial tumor syndromes—*formes frustes* or otherwise.

Reference

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Standardizing gene expression in RT-PCR: is anything normal?

Quantitative RT-PCR is frequently used to identify genes that correlate with tumor diagnosis or disease prognosis. When applying this technique, it is a standard practice to measure the expression of any number of so-called 'housekeeping genes' in order to normalize the data for optimal comparison of expression levels between tissue samples. Housekeeping genes regulate basic and ubiquitous cellular functions, and generally code for components of the cytoskeleton, major histocompatibility complex, glycolytic pathway, protein folding, and synthesis of ribosomal subunits. Currently, the best option seems to be to measure the expression of multiple housekeeping genes and normalize using their mean expression; but this can be impractical when only a small amount of RNA is available from a clinical sample. In this issue of *Lab Invest*, **de Kok *et al***[1] (p. 154) describe the identification of a single housekeeping gene that can replace the measurement of multiple genes. The authors hypothesized that the mean expression of a large set of housekeeping genes with independent cellular functions, in a wide variety of tissue samples, would accurately reflect optimal normalization. Accordingly, RT-PCR was used to measure the expression of 13 ordinarily used housekeeping genes in 80 normal and tumor epithelial tissue samples, which originated from colorectal, breast, prostate, skin, and bladder; and the tumors ranged from noninvasive to metastatic carcinomas. Principal component analysis, linear regression, and difference-plot analysis were used to interpret the data. The authors found that the expression of hypoxanthine ribosyltransferase (HPRT) best reflected the mean expression of a cluster of 11 of the housekeeping genes, and is therefore the most accurate and practical choice as single normalization gene for differential expression studies in pathology research.

Reference

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